Technical Manual

PowerPlex® S5 System

INSTRUCTIONS FOR USE OF PRODUCTS DC6951 AND DC6950.

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Part# TMD021

PowerPlex® S5 System

All technical literature is available on the Internet at: www.promega.com/tbs/
Please visit the web site to verify that you are using the most current version of this Technical Manual.
Please contact Promega Technical Services if you have questions on use of this system.
E-mail: genetic@promega.com

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I. Description

STR (short tandem repeat) loci consist of short, repetitive sequence elements 3–7 base pairs in length (1–4). These repeats are well distributed throughout the human genome and are a rich source of highly polymorphic markers, which may be detected using the polymerase chain reaction (5–8). Alleles of STR loci are differentiated by the number of copies of the repeat sequence contained within the amplified region and are distinguished from one another using radioactive, silver stain or fluorescence detection following electrophoretic separation.

The PowerPlex® S5 System[a–c] allows co-amplification and detection of five loci (four STR loci and Amelogenin), including D8S1179, D18S51, Amelogenin, FGA and TH01. One primer specific for each of Amelogenin, D18S51 and D8S1179 loci is labeled with fluorescein (FL) and one primer specific for each of the TH01 and FGA loci is labeled with 6-carboxy-4´,5´-dichloro-2´,7´-dimethoxy-fluorescein (JOE). All five loci are amplified simultaneously in a single tube and analyzed in a single injection.

The PowerPlex® S5 System is compatible with the ABI PRISM® 310, 3100 and 3100-*Avant* and Applied Biosystems 3130 and 3130*xl* Genetic Analyzers. The protocols presented in this manual were tested at Promega Corporation. Amplification and detection instrumentation may vary. You may need to optimize protocols including cycle number and injection time (or loading volume) for each laboratory instrument. In-house validation should be performed.

The PowerPlex® S5 System provides all of the materials necessary for amplification of STR regions of purified genomic DNA. This manual contains a protocol for use of the PowerPlex® S5 System with the Applied Biosystems 2720 and GeneAmp® PCR system 9600, 9700 and 2400 thermal cyclers in addition to protocols for separation of amplified products and detection of separated material (Figure 1). Protocols for operation of the fluorescence-detection instruments should be obtained from the instrument manufacturer.

Information on other Promega fluorescent STR systems and detection of amplified STR fragments using silver staining is available upon request from Promega or online at: **www.promega.com**

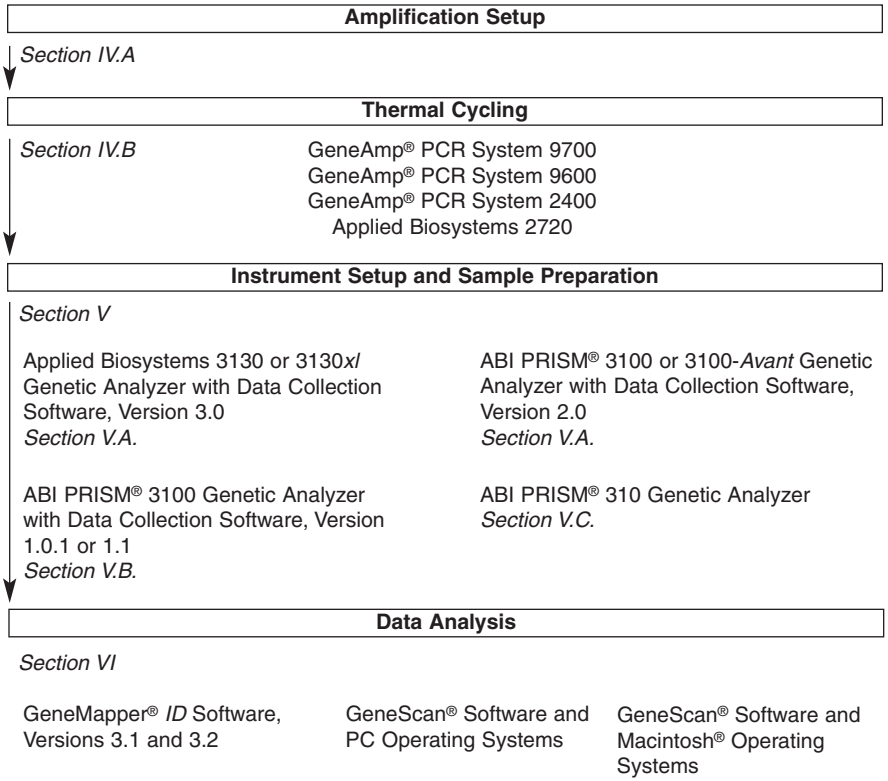

Figure 1. An overview of the PowerPlex® S5 System protocol.

II. Product Components and Storage Conditions

Product	Size	Cat.#
PowerPlex® S5 System	100 reactions	DC6951

Not For Medical Diagnostic Use. Cat.# DC6951 contains sufficient reagents for 100 reactions of 25µl each. Includes:

Pre-amplification Components Box (Blue Label)

500µl	PowerPlex® S5 5X Master Mix
250µl	PowerPlex® S5 10X Primer Pair Mix
25µl	9947A DNA (10ng/µl)
5 × 1,250µl	Water, Amplification Grade

Postamplification Components Box (Beige Label)

25µl	PowerPlex® S5 Allelic Ladder Mix
150µl	Internal Lane Standard (ILS) 600
1	Protocol

II. Product Components and Storage Conditions (continued)

Product	Size	Cat.#
PowerPlex® S5 System	400 reactions	DC6950

Not For Medical Diagnostic Use. Cat.# DC6950 contains sufficient reagents for 400 reactions of 25µl each. Includes:

Pre-amplification Components Box (Blue Label)

4 × 500µl	PowerPlex® S5 5X Master Mix
4 × 250µl	PowerPlex® S5 10X Primer Pair Mix
25µl	9947A DNA (10ng/µl)
10 × 1,250µl	Water, Amplification Grade

Postamplification Components Box (Beige Label)

4 × 25µl	PowerPlex® S5 Allelic Ladder Mix
4 × 150µl	Internal Lane Standard (ILS) 600
1	Protocol

The PowerPlex® S5 Allelic Ladder Mix is provided in a separate, sealed bag for shipping. This component should be moved to the postamplification box after opening. The Water, Amplification Grade, is provided in a separate sealed bag for shipping. This component should be moved to the pre-amplification box after opening.

Storage Conditions: Store all components at –20°C in a nonfrost-free freezer. The PowerPlex® S5 10X Primer Pair Mix, PowerPlex® S5 Allelic Ladder Mix and Internal Lane Standard 600 are light-sensitive and must be stored in the dark. We strongly recommend that pre-amplification and postamplification reagents be stored and used separately with different pipettes, tube racks, etc.

The PowerTyper™ Macro S5, for use with Genotyper® software, can be downloaded at: **www.promega.com/geneticidtools/**

The proper panel and bin files for use with GeneMapper® *ID* software can be obtained from the Promega web site at: **www.promega.com/geneticidtools/panels_bins/**

Matrix standards are required for initial setup of the color separation matrix. The matrix standards are sold separately and are available for the ABI PRISM® 310 Genetic Analyzer (PowerPlex® Matrix Standards, 310; Cat.# DG4640) and the ABI PRISM® 3100 and 3100-*Avant* and Applied Biosystems 3130 and 3130*xl* Genetic Analyzers (PowerPlex® Matrix Standards, 3100/3130; Cat.# DG4650). See Section IX.F for ordering information.

III. Before You Begin

III.A. Precautions

The application of PCR-based typing for forensic or paternity casework requires validation studies and quality-control measures that are not contained in this manual (9–11). The quality of purified DNA, as well as small changes in buffers, ionic strength, primer concentrations, choice of thermal cycler and thermal cycling conditions, can affect PCR success. We suggest strict adherence to recommended procedures for amplification, as well as fluorescence detection.

PCR-based STR analysis is subject to contamination by very small amounts of nontemplate human DNA. Extreme care should be taken to avoid cross-contamination when preparing sample DNA, handling primer pairs, assembling amplification reactions and analyzing amplification products. Reagents and materials used prior to amplification (PowerPlex® S5 5X Master Mix, PowerPlex® S5 10X Primer Pair Mix, Water, Amplification Grade, and 9947A DNA) are provided in a separate box and should be stored separately from those used following amplification (PowerPlex® S5 Allelic Ladder Mix and Internal Lane Standard 600). Always include a negative control reaction (i.e., no template) to detect reagent contamination. We highly recommend the use of gloves and aerosol-resistant pipette tips (e.g., ART® tips, Section IX.F).

III.B. Matrix Standardization or Spectral Calibration

Proper generation of a matrix file is critical to evaluate multicolor systems with the ABI PRISM® 310, 3100 and 3100-*Avant* and Applied Biosystems 3130 and 3130*xl* Genetic Analyzers. A matrix must be generated for each individual instrument.

The PowerPlex® Matrix Standards, 310 (Cat.# DG4640), is required for matrix standardization for the ABI PRISM® 310 Genetic Analyzer. For best results, the PowerPlex® Matrix Standards, 3100/3130 (Cat.# DG4650), should not be used to generate a matrix on the ABI PRISM® 310 Genetic Analyzer.

The PowerPlex® Matrix Standards, 3100/3130 (Cat.# DG4650), is required for spectral calibration on the ABI PRISM® 3100 and 3100-*Avant* Genetic Analyzers and Applied Biosystems 3130 and 3130*xl* Genetic Analyzers. The PowerPlex® Matrix Standards, 310 (Cat.# DG4640), cannot be used to generate a matrix on these instruments.

For protocols and additional information on matrix standardization, see the *PowerPlex® Matrix Standards, 310, Technical Bulletin* #TBD021, which is supplied with Cat.# DG4640. For protocols and additional information about spectral calibration, see the *PowerPlex® Matrix Standards, 3100/3130, Technical Bulletin* #TBD022, which is supplied with Cat.# DG4650. These manuals are available upon request from Promega or online at: **www.promega.com/tbs/**

IV. Protocols for DNA Amplification Using the PowerPlex® S5 System

Materials to Be Supplied by the User

- Applied Biosystems 2720 or GeneAmp® PCR System 9600, 9700 or 2400 thermal cyclers (Applied Biosystems)
- microcentrifuge
- 0.2ml MicroAmp® reaction tubes or MicroAmp® optical 96-well reaction plate (Applied Biosystems)
- 1.5ml amber-colored microcentrifuge tubes (Fisher Cat.# 05-402-26)
- aerosol-resistant pipette tips (Section IX.F)

We routinely amplify 0.25–0.5ng of template DNA in a 25µl reaction volume using the protocols detailed below. Saturated peaks may be observed if more than the recommended amount of template is used. When using high template amounts, reduce the amount of template DNA or the number of cycles (25–28 cycles).

The PowerPlex® S5 System is optimized for the GeneAmp® PCR System 9700 thermal cycler. Amplification protocols for the Applied Biosystems 2720 and GeneAmp® PCR Systems 9600 and 2400 thermal cyclers are provided.

IV.A. Amplification Setup

The use of gloves and aerosol-resistant pipette tips is highly recommended to prevent cross-contamination. Keep all pre-amplification and postamplification reagents in separate rooms. Prepare amplification reactions in a room dedicated for reaction setup. Use equipment and supplies dedicated for amplification setup.

(!) Meticulous care must be taken to ensure successful amplification. A guide to amplification troubleshooting is provided in Section VII.A.

1. Thaw the PowerPlex® S5 5X Master Mix, PowerPlex® S5 10X Primer Pair Mix and 9947A DNA completely.

   Note: Mix reagents by vortexing each tube for 15 seconds before each use. Do not centrifuge the 10X Primer Pair Mix, as this may cause the primers to be concentrated at the bottom of the tube.

2. Determine the number of reactions to be set up. This should include positive and negative control reactions. Add 1 or 2 reactions to this number to compensate for pipetting error. While this approach does waste a small amount of each reagent, it ensures that you will have enough PCR amplification mix for all samples. It also ensures that each reaction contains the same PCR amplification mix.

3. Place one clean, 0.2ml amplification tube for each reaction into a rack, and label appropriately. Alternatively, use a MicroAmp® plate, and label appropriately.

4. Add the final volume of each reagent listed in Table 1 into a sterile, 1.5ml amber-colored tube. Mix gently.

   Table 1 shows the component volumes per reaction. A worksheet to calculate the required amount of each component of the PCR amplification mix is provided in Section IX.D (Table 5).

   Note: In tests performed at Promega, we have found that reactions can remain at room temperature for up to 4 hours after reaction assembly and prior to thermal cycling with no adverse effect on amplification results.

Table 1. PCR Amplification Mix for the PowerPlex® S5 System.

PCR Amplification Mix Component[1]	Volume Per Reaction
Water, Amplification Grade	to a final volume of 25.0µl
PowerPlex® S5 5X Master Mix	5.0µl
PowerPlex® S5 10X Primer Pair Mix	2.5µl
template DNA (0.25–0.5ng)[2]	up to 17.5µl
total reaction volume	**25µl**

[1]Add Water, Amplification Grade, to the PCR amplification mix first, then add PowerPlex® S5 5X Master Mix and PowerPlex® S5 10X Primer Pair Mix. The template DNA will be added at Step 7.

[2]Store DNA templates in Water, Amplification Grade, or TE^{-4} buffer (10mM Tris-HCl [pH 8.0], 0.1mM EDTA). If the DNA template is stored in TE buffer that is not pH 8.0 or contains a higher EDTA concentration, the volume of the DNA sample added should not exceed 20% of the final reaction volume. PCR amplification efficiency and quality can be greatly altered by changes in pH (due to added Tris-HCl), available magnesium concentration (due to chelation by EDTA) or other PCR inhibitors, which may be present at low concentrations depending on the source of the template DNA and the extraction procedure used.

5. Vortex the PCR amplification mix for 5–10 seconds.

Note: Failure to vortex the PCR amplification mix sufficiently can result in poor amplification, peak height imbalance and extra peaks in the range of 50–80bp.

6. Pipet the appropriate volume of PCR amplification mix into each reaction tube.

7. Pipet the template DNA (0.25–0.5ng) for each sample into the respective tube containing PCR amplification mix.

8. For the positive amplification control, dilute 9947A DNA from 10ng/µl to 0.5ng in the desired template DNA volume. Pipet 0.5ng of diluted 9947A DNA into a reaction tube containing PCR amplification mix.

9. For the negative amplification control, pipet Water, Amplification Grade, (instead of template DNA) into a reaction tube containing PCR amplification mix.

Remove any air bubbles from the bottom of the tubes by careful pipetting or briefly centrifuging the tubes or plate. Failure to remove air bubbles may result in inconsistent results.

IV.B. Amplification Thermal Cycling

Amplification and detection instrumentation may vary. You may need to optimize protocols including cycle number and injection time for each laboratory instrument. Testing at Promega Corporation shows that 30 cycles work well with 0.25–0.5ng of purified DNA. Increased cycle number (32–34 cycles) will result in increased sensitivity when using low amounts of template. Decreased cycle number may be used if a higher amount of template is added to the amplification. For reactions containing ≥1ng of DNA the number of cycles may be reduced (25–28 cycles). In-house validation should be performed.

1. Place the tubes or MicroAmp® plate in a thermal cycler.
2. Run the recommended protocol provided below for use with the GeneAmp® PCR System 9600, 9700 and 2400 thermal cyclers and Applied Biosystems 2720 thermal cycler.

   For information about other thermal cyclers, please contact Promega Technical Services by e-mail: **genetic@promega.com**

Thermal Cycling Protocol[1]
96°C for 2 minutes, then:
94°C for 30 seconds 60°C for 2 minutes 72°C for 90 seconds for 30 cycles, then:
60°C for 45 minutes
4°C soak

[1]When running the GeneAmp® PCR System 9700 thermal cycler, use the Method Option, Ramp Speed: 9600.

3. After completion of the thermal cycling protocol, store samples at –20°C in a light-protected box.

Notes:

1. Storage of amplified samples at 4°C or higher may produce degradation products.
2. A precipitate may form during amplification. This precipitate does not affect downstream analysis or capillary electrophoresis performance.

V. Instrument Setup and Sample Preparation

V.A. Detection of Amplified Fragments Using the ABI PRISM® 3100 or 3100-*Avant* Genetic Analyzer with Data Collection Software, Version 2.0, and the Applied Biosystems 3130 or 3130*xl* Genetic Analyzer with Data Collection Software, Version 3.0

Materials to Be Supplied by the User

- dry heating block, water bath or thermal cycler
- crushed ice or ice-water bath
- centrifuge compatible with 96-well plates
- aerosol-resistant pipette tips
- 3100 or 3130 capillary array, 36cm
- performance optimized polymer 4 (POP-4™) for the 3100 or 3130
- 10X genetic analyzer buffer with EDTA
- MicroAmp® optical 96-well plate and septa
- Hi-Di™ formamide (Applied Biosystems Cat.# 4311320)
- PowerPlex® Matrix Standards, 3100/3130 (Cat.# DG4650)

The quality of formamide is critical. Use Hi-Di™ formamide with a conductivity less than 100µS/cm. Freeze formamide in aliquots at –20°C. Multiple freeze-thaw cycles or long-term storage at 4°C may cause a breakdown of the formamide. Formamide with a conductivity greater than 100µS/cm may contain ions that compete with DNA during injection, which results in lower peak heights and reduced sensitivity. A longer injection time may not increase the signal.

Caution: Formamide is an irritant and a teratogen; avoid inhalation and contact with skin. Read the warning label and take appropriate precautions when handling this substance. Always wear gloves and safety glasses when working with formamide.

Sample Preparation

1. Prepare a loading cocktail by combining and mixing Internal Lane Standard 600 and Hi-Di™ formamide as follows:

   [(0.5µl ILS 600) × (# injections)] + [(9.5µl Hi-Di™ formamide) × (# injections)]

   Note: The volume of internal lane standard used in the loading cocktail can be increased or decreased to adjust the intensity of size standard peaks. The optimal peak height for the 100-base fragment of the internal lane standard is 500–1,000RFU. If peak heights are too low, we recommend altering the formamide/internal lane standard mix to contain 1.0µl of ILS 600 and 9.0µl of Hi-Di™ formamide. If peak heights are too high, we recommend altering the loading cocktail to contain 0.25µl of ILS 600 and 9.75µl of formamide.

2. Mix for 10–15 seconds using a vortex mixer.

3. Pipet 10µl of formamide/internal lane standard mix into each well.

V.A. Detection of Amplified Fragments Using the ABI PRISM® 3100 or 3100-*Avant* Genetic Analyzer with Data Collection Software, Version 2.0, and the Applied Biosystems 3130 or 3130*xl* Genetic Analyzer with Data Collection Software, Version 3.0 (continued)

4. Add 1µl of amplified sample (or 1µl of allelic ladder mix). Cover wells with appropriate septa.

   Notes:

   1. Instrument detection limits vary; therefore, the injection time, voltage or amount of product mixed with loading cocktail may need to be increased or decreased. Use the "Module Manager" in the Tools menu to modify the injection time or voltage in the run module.
   2. A precipitate may form during amplification. This precipitate does not affect downstream analysis or capillary electrophoresis performance.

5. Centrifuge plate briefly to remove air bubbles from the wells if necessary.

6. Denature samples at 95°C for 3 minutes, then immediately chill on crushed ice or in an ice-water bath for 3 minutes. Denature samples just prior to loading the instrument.

Instrument Preparation

Refer to the instrument manual for instructions on cleaning the pump blocks, installing the capillary array, performing a spatial calibration and adding polymer to the reserve syringe.

Analyze samples as described in the user's manual for the ABI PRISM® 3100 or 3100-*Avant* Genetic Analyzer with data collection software, version 2.0, and the Applied Biosystems 3130 or 3130*xl* Genetic Analyzer with the following exceptions.

1. In the Module Manager, select "New". Select "Regular" in the Type drop-down list, and select "HIDFragmentAnalysis36_POP4" in the Template drop-down list. Confirm that the injection time is 5 seconds and the injection voltage is 3kV. Change the run time to 1,200 seconds. Give a new name to your run module, and select "OK".

   Note: Instrument sensitivities can vary. The injection time and voltage may be adjusted in the Module Manager. A suggested range for the injection time is 3–15 seconds and for the injection voltage is 1–5kV.

2. In the Protocol Manager, select "New". Type a name for your protocol. Select "Regular" in the Type drop-down list, and select the run module you created in the previous step in the Run Module drop-down list. Lastly, select "F" in the Dye-Set drop-down list. Select "OK".

3. In the Plate Manager, create a new plate record as described in the instrument user's manual. In the dialog box that appears, select "GeneMapper—Generic" in the Application drop-down list, and select the appropriate plate type (96-well). Add entries in the owner and operator windows, and select "OK".

   Note: If autoanalysis of sample data is desired, refer to the instrument user's manual for instructions.

4. In the GeneMapper® plate record, enter sample names in the appropriate cells. Scroll to the right. In the results group 1 column, select the desired results group. In the instrument protocol 1 column, select the protocol you created in Step 2. Be sure this information is present for each row that contains a sample name. Select "OK".

   Note: To create a new results group, select "New" in the drop-down menu in the results group column. Select the General tab, and enter a name. Select the Analysis tab, and select "GeneMapper—Generic" in the Analysis type drop-down list.

5. Place samples in the instrument, and close the instrument doors.

6. In the spectral viewer, confirm that dye set F is active, and set the correct active calibration for dye set F.

7. In the run scheduler, locate the plate record that you just created in Steps 3 and 4, and click once on the name to highlight it.

8. Once the plate record is highlighted, click the plate graphic that corresponds to the plate on the autosampler that contains your amplified samples.

9. When the plate record is linked to the plate, the plate graphic will change from yellow to green, and the green Run Instrument arrow becomes enabled.

10. Click the green Run Instrument arrow on the toolbar to start the sample run.

11. Monitor electrophoresis by observing the run, view, array or capillaries viewer windows in the collection software. Each injection will take approximately 35 minutes.

V.B. Detection of Amplified Fragments Using the ABI PRISM® 3100 Genetic Analyzer with Data Collection Software, Version 1.0.1 or 1.1

Materials to Be Supplied by the User

- 95°C dry heating block, water bath or thermal cycler
- crushed ice or ice-water bath
- aerosol-resistant pipette tips
- centrifuge compatible with 96-well plates
- 3100 capillary array, 36cm
- performance optimized polymer 4 (POP-4™) for the 3100
- 10X genetic analyzer buffer with EDTA
- MicroAmp® optical 96-well plate and septa for the 3100
- Hi-Di™ formamide (Applied Biosystems Cat.# 4311320)
- PowerPlex® Matrix Standards, 3100/3130 (Cat.# DG4650)

The quality of formamide is critical. Use Hi-Di™ formamide with a conductivity less than 100µS/cm. Freeze formamide in aliquots at –20°C. Multiple freeze-thaw cycles or long-term storage at 4°C may cause a breakdown of the formamide. Formamide with a conductivity greater than 100µS/cm may contain ions that compete with DNA during injection, which results in lower peak heights and reduced sensitivity. A longer injection time may not increase the signal.

Caution: Formamide is an irritant and a teratogen; avoid inhalation and contact with skin. Read the warning label, and take appropriate precautions when handling this substance. Always wear gloves and safety glasses when working with formamide.

Sample Preparation

1. Prepare a loading cocktail by combining and mixing Internal Lane Standard 600 and Hi-Di™ formamide as follows:

   [0.5µl ILS 600) × (# injections)] + [(9.5µl Hi-Di™ formamide) × (# injections)]

   Note: The volume of internal lane standard used in the loading cocktail can be increased or decreased to adjust the intensity of size standard peaks. The optimal peak height for the 100-base fragment of the internal lane standard is 500–1,000RFU. If peak heights are too low, we recommend altering the formamide/internal lane standard mix to contain 1.0µl of ILS 600 and 9.0µl of Hi-Di™ formamide. If peak heights are too high, we recommend altering the loading cocktail to contain 0.25µl of ILS 600 and 9.75µl of formamide.

2. Mix for 10–15 seconds using a vortex mixer.

3. Pipet 10µl of formamide/internal lane standard mix into each well.

4. Add 1µl of amplified sample (or 1µl of allelic ladder mix). Cover wells with appropriate septa.

   Notes:

   1. Instrument detection limits vary; therefore, injection time or the amount of product mixed with loading cocktail may need to be increased or decreased. Use the Module Editor in the Tools menu to modify the injection time or voltage in the run module.
   2. A precipitate may form during amplification. This precipitate does not affect downstream analysis or capillary electrophoresis performance.

5. Centrifuge plate briefly to remove air bubbles from the wells if necessary.

6. Denature samples at 95°C for 3 minutes, then immediately chill on crushed ice or in an ice-water bath for 3 minutes. Denature samples just prior to loading the instrument.

Instrument Preparation

Refer to the *ABI PRISM® 3100 Genetic Analyzer User's Manual* for instructions on cleaning the blocks, installing the capillary array, performing a spatial calibration and adding polymer to the reserve syringe.

1. Open the ABI PRISM® 3100 data collection software.

2. Change the "GeneScan36_POP4DefaultModule" module run time to 1,200 seconds.

3. Change the injection voltage to 3kV.

4. Change the injection time to 11 seconds.

   Note: Instrument sensitivities can vary. The injection time and voltage may be adjusted in the Module Manager. A suggested range for the injection time is 3–22 seconds and for the injection voltage is 1–3kV.

5. Save the module with a new name (e.g., GeneScan36_POP4PowerPlexS5_3kV_11secs_1200). Use this as the initial run module for all runs.

6. Open a new plate record. Name the plate, and select "GeneScan". Select the plate size (96-well). Select "Finish".

7. Complete the plate record spreadsheet for the wells you have loaded. Enter appropriate information into the sample name and color info columns. For allelic ladder samples, insert the word "ladder" into the color info column for the blue and green dye colors. This information must be entered to successfully analyze data with the PowerTyper™ S5 Macro.

8. In the BioLIMS Project column, select "3100_Project1" from the pull-down menu.

9. In the Dye Set column, select "Z" from the pull-down menu.

V.B. Detection of Amplified Fragments Using the ABI PRISM® 3100 Genetic Analyzer with Data Collection Software, Version 1.0.1 or 1.1 (continued)

10. When using the ABI PRISM® 3100 data collection software version 1.0.1 or 1.1, select "GeneScan36_POP4PowerPlexS5_3kV_11secs_1200" from the pull-down menu in the Run Module 1 column.
11. To collect the data without autoanalyzing, select "No Selection" in the Analysis Module 1 column. Analysis parameters can be applied after data collection and during data analysis using the GeneScan® analysis software.
12. Select "OK". This new plate record will appear in the pending plate records table on the plate setup page of the collection software.
13. Place samples in the instrument, and close the instrument doors.
14. Locate the pending plate record that you just created, and click once on the name.
15. Once the pending plate record is highlighted, click on the plate graphic that corresponds to the plate on the autosampler that contains your amplified samples to link the plate to the plate record.
16. When the plate record is linked to the plate, the plate graphic will change from yellow to green, the plate record moves from the pending plate records table to the linked plate records table, and the Run Instrument button becomes enabled.
17. Select the Run Instrument button on the toolbar to start the sample run.
18. Monitor electrophoresis by observing the run, status, array and capillary views windows in the collection software. Each injection will take approximately 35 minutes.

V.C. Detection of Amplified Fragments Using the ABI PRISM® 310 Genetic Analyzer

Materials to Be Supplied by the User

- 95°C dry heating block, water bath or thermal cycler
- 310 capillaries, 47cm × 50µm
- performance optimized polymer 4 (POP-4™) for the 310
- 10X genetic analyzer buffer with EDTA
- sample tubes and septa
- aerosol-resistant pipette tips
- Hi-Di™ formamide (Applied Biosystems Cat.# 4311320)
- PowerPlex® Matrix Standards, 310 (Cat.# DG4640)
- crushed ice or ice-water bath

The quality of formamide is critical. Use Hi-Di™ formamide with a conductivity less than 100µS/cm. Freeze formamide in aliquots at –20°C. Multiple freeze-thaw cycles or long-term storage at 4°C may cause a breakdown of the formamide. Formamide with a conductivity greater than 100µS/cm may contain ions that compete with DNA during injection, which results in lower peak heights and reduced sensitivity. A longer injection time may not increase the signal.

Caution: Formamide is an irritant and a teratogen; avoid inhalation and contact with skin. Read the warning label and take appropriate precautions when handling this substance. Always wear gloves and safety glasses when working with formamide.

Sample Preparation

1. Prepare a loading cocktail by combining Internal Lane Standard 600 and Hi-Di™ formamide as follows:

   [(1.0µl ILS 600) × (# injections)] + [(24.0µl Hi-Di™ formamide) × (# injections)]

   Note: The volume of internal lane standard used in the loading cocktail can be increased or decreased to adjust the intensity of size standard peaks. If peak heights are too high, we recommend altering the formamide/internal lane standard mix to contain 0.5µl of ILS 600 and 24.5µl of Hi-Di™ formamide.

2. Mix for 10–15 seconds using a vortex mixer.

3. Combine 25.0µl of prepared loading cocktail with 1.0µl of amplified sample or 1.0µl of PowerPlex® S5 Allelic Ladder Mix.

   Notes:

   1. Instrument detection limits vary; therefore, injection time or the amount of product mixed with loading cocktail may need to be increased or decreased.
   2. A precipitate may form during amplification. This precipitate does not affect downstream analysis or capillary electrophoresis performance.

4. Denature samples and ladder by heating at 95°C for 3 minutes, and immediately chill on crushed ice or in an ice-water bath for 3 minutes. Denature samples just prior to loading.

5. Assemble the tubes in the appropriate autosampler tray (48- or 96-tube).

6. Place the autosampler tray in the instrument, and close the instrument doors.

V.C. Detection of Amplified Fragments Using the ABI PRISM® 310 Genetic Analyzer (continued)

Instrument Preparation

Refer to the *ABI PRISM® 310 Genetic Analyzer User's Manual* for instructions on cleaning the pump block, installing the capillary, calibrating the autosampler and adding polymer to the syringe.

1. Open the ABI PRISM® 310 data collection software.
2. Prepare a GeneScan® sample sheet as described in the *ABI PRISM® 310 Genetic Analyzer User's Manual*. Enter the appropriate sample information in the sample info column.

   For rows containing PowerPlex® S5 Allelic Ladder Mix, insert the word "ladder" in the sample info column for the blue dye color and green dye color. This information must be entered to successfully analyze your data using the PowerTyper™ S5 Macro.
3. Create a new GeneScan® injection list. Select the appropriate sample sheet by using the pull-down menu.
4. Select the "GS STR POP4 (1ml) A" Module using the pull-down menu. Change the injection time to the appropriate setting and the run time to 23 minutes. Keep the settings for the remaining parameters as shown below:

Inj. Secs:	2–5
Inj. kV:	15.0
Run kV:	15.0
Run °C:	60
Run Time:	23

   You may need to optimize the injection time for individual instruments. We recommend injection times of 2–5 seconds.

   Note: Migration of fragments may vary slightly over the course of a long ABI PRISM® 310 Genetic Analyzer run. This may be due to changes in temperature or changes in the column. When analyzing many samples, injections of allelic ladder at different times throughout the run can aid in accurately genotyping samples.
5. Select the appropriate matrix file (Section III.B).
6. To analyze data automatically, select the auto analyze checkbox and the appropriate analysis parameters and size standard. Refer to the *ABI PRISM® 310 Genetic Analyzer User's Manual* for specific information on these options.
7. After loading the sample tray and closing the doors, select "Run" to start the capillary electrophoresis (CE) system.
8. Monitor electrophoresis by observing the raw data and status windows. Each sample will take approximately 35 minutes for syringe pumping, sample injection and sample electrophoresis.

VI. Data Analysis

VI.A. PowerPlex® Panel and Bin Sets with GeneMapper® *ID*, Version 3.2

To facilitate analysis of data generated with the PowerPlex® S5 System, we have created panel and bin files to allow automatic assignment of genotypes using GeneMapper® *ID* software, version 3.2. We recommend that users of GeneMapper® *ID* software, version 3.2, complete the *Applied Biosystems GeneMapper®* ID *Software Human Identification Analysis Tutorial* to familiarize themselves with the proper operation of the software. For GeneMapper® *ID* software, version 3.1, users we recommend upgrading to version 3.2.

Getting Started

1. Obtain the proper panel and bin files for use with GeneMapper® *ID* from the Promega web site at: **www.promega.com/geneticidtools/panels_bins/**
2. Enter your contact information, and select "GeneMapper ID version 3.2". Select "Submit".
3. Select the "PowerPlex® S5 Panels & Bin Sets" link, and save the .zip file to your computer.
4. Open the files using the Windows® WinZip program, and save the unzipped files to a known location on your computer.

Importing Panel and Bin Files

These instructions loosely follow the Applied Biosystems GeneMapper® *ID* software tutorial, pages 1–4.

1. Open the GeneMapper® *ID* software, version 3.2.
2. Select "Tools", then "Panel Manager".
3. Highlight the Panel Manager icon in the upper left tile (navigation pane).
4. Select "File", then "Import Panels".
5. Navigate to the saved panel and bin files. Select "PowerPlex_S5_Panels_ID3.2x.txt".
6. In the navigation pane, highlight the PowerPlex_S5_Panels folder that you just imported.
7. Select "File", then "Import Bin Set".
8. Navigate to the saved panel and bin files. Select "PowerPlex_S5_Bins_ID3.2x.txt", then "Import".
9. At the bottom of the panel manager window, select "Apply", then "OK". The Panel Manager window will close automatically.

VI.B. Creating an Analysis Method with GeneMapper® *ID* Software

These instructions loosely follow the Applied Biosystems GeneMapper® *ID* software tutorial, pages 1–11.

1. Select "Tools", then "GeneMapper Manager".
2. Select the Analysis Methods tab.
3. Select "New", and a new analysis method dialog box will open.
4. Select "HID", and select "OK".

   Note: If you do not see the HID option, you do not have the GeneMapper® *ID* software. Contact Applied Biosystems.
5. Enter a descriptive name for the analysis method, such as "PowerPlexS5 advanced".
6. Select the Allele tab (Figure 2).
7. Select the bin set corresponding to the PowerPlex® System "PowerPlex_S5_Bin_ID3.2x".
8. Ensure that the "Use marker-specific stutter ratio if available" box is checked.

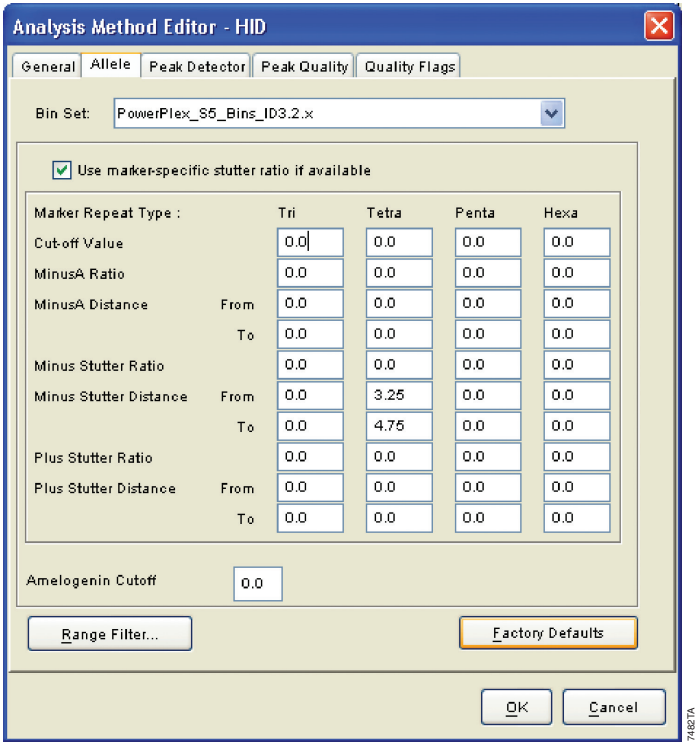

Figure 2. The Allele tab. Select the bin set "PowerPlex_S5_Bins_ID3.2x".

9. Enter the values shown in Figure 2 for proper filtering of stutter peaks when using the PowerPlex® Systems. For an explanation of the proper usage and effects of these settings, refer to the Applied Biosystems user bulletin titled "*Installation Procedures and New Features for GeneMapper* ID *Software 3.2*" and the "*GeneMapper®* ID *Software Version 3.1 Human Identification Analysis User Guide*.

   Note: Some of these settings have been optimized and are different from the recommended settings in the user bulletin.

10. Select the Peak Detector tab. We recommend the settings shown in Figure 3.

    Note: Select full range or partial range for the analysis range. When using a partial range, choose an appropriate analysis range based on the data. Choose a start point after the primer peak and just before the first defined internal lane standard peak to help ensure proper sizing of the internal lane standard.

11. Select the Peak Quality tab. You may change the settings for peak quality.

    Note: For Steps 11 and 12, see the GeneMapper® *ID* user's manual for more information.

12. Select the Quality Flags tab. You may also change these settings.

13. Select "OK" to save your settings.

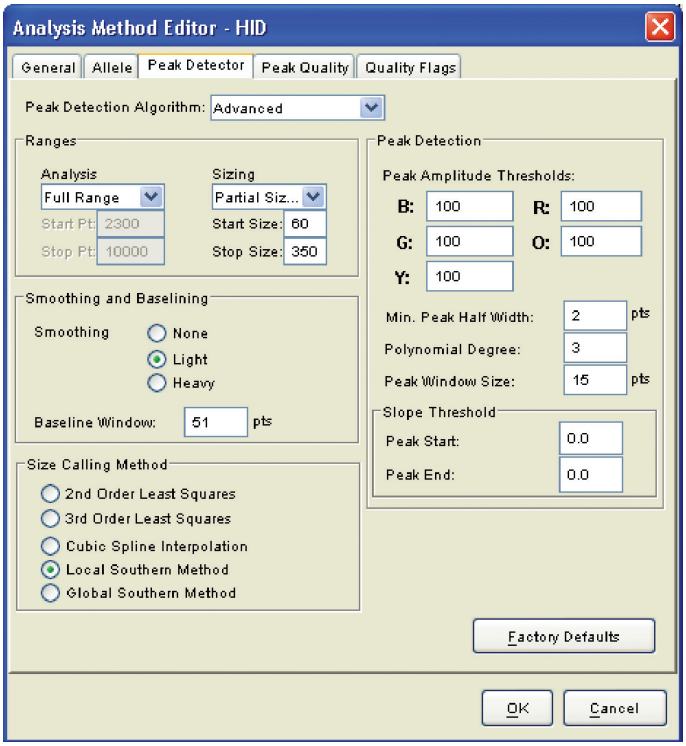

Figure 3. The Peak Detector tab.

Creating a Size Standard

1. Select "Tools", then "GeneMapper Manager".
2. Select the Size Standard tab.
3. Select "New".
4. Select "Basic or Advanced" (Figure 4). The type of analysis method selected must match the type of analysis method created earlier. Select "OK".
5. Enter a detailed name such as "60–350 ILS Adv" in the "Size Standard Editor" (Figure 5).
6. Choose red as the color for the size standard dye.
7. Enter sizes for the 60–350bp internal lane standard fragments (Section IX.C, Figure 11).

   Note: With the run times recommended in this manual, not all ILS 600 fragments will be detected. Label all fragments present. For accurate sizing, the 350bp fragment must be detected. If present, larger fragments also may be labeled.
8. Select "OK".

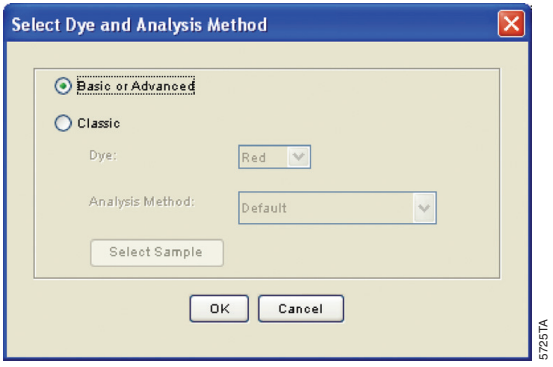

Figure 4. The Select Dye and Analysis Method window.

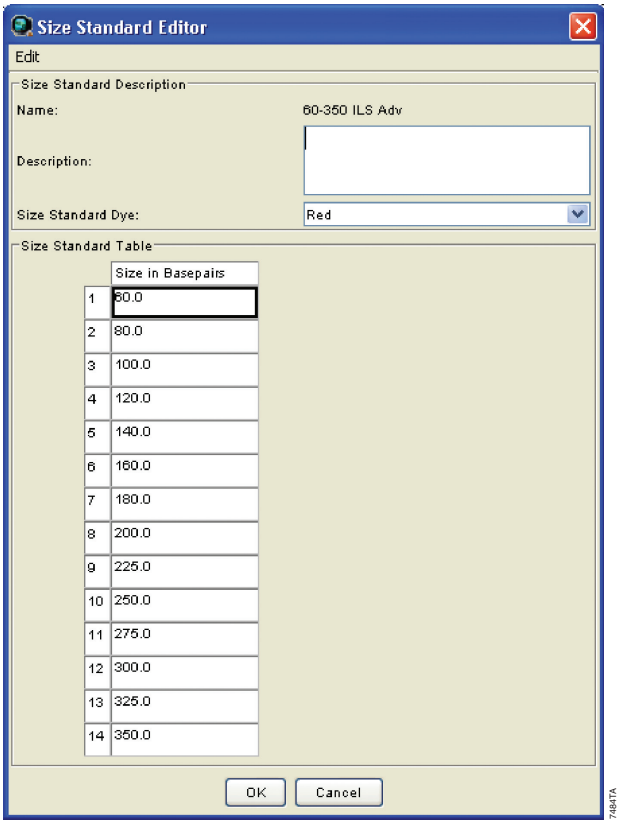

Figure 5. The Size Standard Editor.

Processing Sample Data

1. Import sample files into a new project as described in the *Applied Biosystems GeneMapper® ID Software Human Identification Analysis Tutorial.*
2. In the Sample Type column, use the drop-down menu to select "Ladder", "Sample", "Positive Control" or "Negative Control". Every folder in the project must contain at least one ladder that is designated as such for proper genotyping.
3. In the Analysis Method column, select the analysis method created earlier.
4. In the Panel column, select "PowerPlex_S5_Panels". This is the panel set that was imported in Section VI.A.
5. In the Size Standard column, select the size standard that was created in Creating a Size Standard section.
6. If analyzing data from an ABI PRISM® 310 Genetic Analyzer, ensure that the appropriate matrix file is selected in the Matrix column.
7. Select "Analyze" (green arrow button) to start data analysis.

VI.C. Sample Analysis Using the GeneScan® Software and PC Operating Systems

1. Analyze data using the GeneScan® analysis software.
2. Review the raw data for one or more sample runs. Highlight the sample file name, then in the Sample menu, select "raw data". Move the cursor so that the crosshair is on the baseline to the right of the large primer peak (before the first internal lane standard peak [red]). Use the X-value number shown at the bottom left of the window for the start position in the analysis parameters.
3. The recommended analysis parameters are shown in Figure 6.
4. The analysis parameters can be saved in the Params folder; in most installations this is located at:
   C:\AppliedBio\Shared\Analysis\Sizecaller\Params\
5. Apply the stored analysis parameters file to the samples.

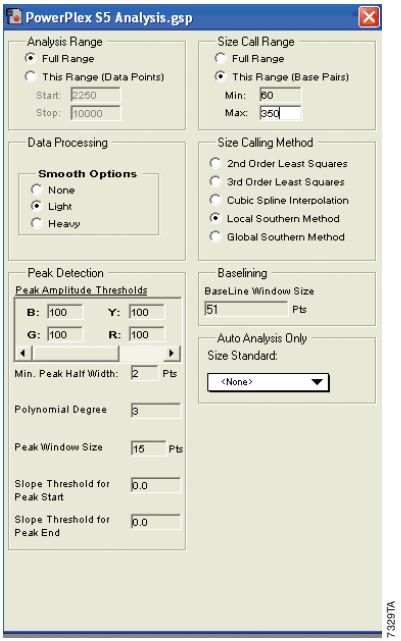

Figure 6. The analysis parameters window. The start point of the analysis range, which will vary, is defined in Section VI.C or VI.D, Step 2.

6. Assign a new size standard. Select a sample file, and highlight the arrow next to size standard. Select "define new". Assign the size standard peaks as shown in Figure 11 in Section IX.C. Store the size standard in the Size Standards folder at:
C:\AppliedBio\Shared\Analysis\Sizecaller\SizeStandards\

   Note: With the run times recommended in this manual, not all ILS 600 fragments will be detected. Label all fragments present. For accurate sizing, the 350bp fragment must be detected. If present, larger fragments may be labeled also.

7. Apply the size standard file to the samples, then analyze the sample files. See Section VI.E for additional information on the use of the PowerTyper™ S5 Macro and Genotyper® software.

   For additional information regarding the GeneScan® analysis software, refer to the *GeneScan® Analysis Software User's Manual*.

VI.D. Sample Analysis Using the GeneScan® Software and Macintosh® Operating Systems

1. Analyze data using the GeneScan® analysis software.
2. Review the raw data for one or more sample runs. Highlight the sample file name, then in the Sample menu, select "raw data". Move the cursor so that the crosshair is on the baseline to the right of the large primer peak (before the first internal lane standard peak [red]). Use the X-value number shown at the bottom left of the window for the start position in the analysis parameters.
3. The recommended analysis parameters are:

Analysis Range	Start: Defined in Step 2 Stop: 10,000
Data Processing	Baseline: Checked Multicomponent: Checked Smooth Options: Light[1]
Peak Detection	Peak Amplitude Thresholds[2]: B: Y: G: R: Min. Peak Half Width: 2pts
Size Call Range	Min: 60 Max: 350
Size Calling Method	Local Southern Method
Split Peak Correction	None

[1]Smooth options should be determined by individual laboratories.

[2]The peak amplitude thresholds are the minimum peak heights that the software will call as a peak. Values for the peak amplitude thresholds are usually 50–200RFU and should be determined by individual laboratories.

VI.D. Sample Analysis Using the GeneScan® Software and Macintosh® Operating Systems (continued)

4. The analysis parameters can be saved in the Params folder.
5. Apply the stored analysis parameters file to the samples.
6. Assign a new size standard. Select a sample file, highlight the arrow next to size standard, then select "define new". Assign the size standard peaks as shown in Figure 11 in Section IX.C. Store the size standard in the Size Standards folder.

   Note: With the run times recommended in this manual, not all ILS 600 fragments will be detected. Label all fragments present. For accurate sizing, the 350bp fragment must be detected. If present, larger fragments may be labeled also.
7. Apply the size standard file to the samples, then analyze the sample files. See Section VI.E for additional information on the use of the PowerTyper™ S5 Macro and Genotyper® software.

   For additional information regarding the GeneScan® analysis software, refer to the *GeneScan® Analysis Software User's Manual*.

VI.E. Sample Analysis Using the Genotyper® Software and PowerTyper™ S5 Macro

To facilitate analysis of data generated with the PowerPlex® S5 System, we have created a file to allow automatic assignment of genotypes using the Genotyper® software. After samples are amplified, detected using the ABI PRISM® 310 or 3100 Genetic Analyzer (using data collection software, version 1.0.1 or 1.1) and analyzed using the GeneScan® analysis software, sample files can be imported into the Genotyper® program and analyzed using the PowerTyper™ S5 Macro.

The PowerTyper™ S5 Macro can be downloaded from the Promega web site at: **www.promega.com/geneticidtools/**

The PowerTyper™ S5 Macro is used in conjunction with Macintosh® Genotyper® software, version 2.5, and Windows NT® Genotyper® software, version 3.6, or later. The Genotyper® software must be installed on your computer before the PowerTyper™ S5 Macro can be used.

Be certain the sample info (Macintosh® computers) or color info (Windows NT® operating systems) column for each lane containing allelic ladder mix contains the word "ladder". The macro uses the word "ladder" to identify the sample file(s) containing allelic ladder. Sample info can be added or modified after importing into the PowerTyper™ Macro. Highlight the sample, then select "show dye/lanes window" in the Views menu.

1. Download the PowerTyper™ S5 Macro from the Promega web site.
2. Open the Genotyper® software, then the PowerTyper™ S5 Macro. For questions about the Genotyper® software, refer to the *Genotyper® Analysis Software User's Manual*.

3. In the File menu, select "Import", and import the GeneScan® project or sample files to be analyzed. Import the blue, green and red dye colors.

   Note: To select the dye colors to be imported, select "Set Preferences" in the Edit menu.

4. Double-click on the Check ILS macro. The macros are listed at the bottom left corner of the active window. A plots window will be displayed to show the internal lane standard (i.e., ILS 600) in the red dye color. Scroll down to view and confirm that internal lane standard fragment sizes are correct. If necessary, redefine internal lane standard fragments and re-analyze samples using the GeneScan® software.

   Note: The software uses one ladder sample to determine allele sizes. The macro uses the first ladder sample imported for allele designations.

5. Double-click on the POWER macro. The POWER macro identifies alleles in the ladder sample and calculates offsets for all loci. This process may take several minutes. When completed, a plots window will open to display the allelic ladders (i.e., D8S1179, D18S51, Amelogenin, etc.).

   In general, allelic ladders contain fragments of the same lengths as many known alleles for the locus. Allelic ladder sizes and repeat units are listed in Table 3 (Section IX.A). Analysis using GeneScan® analysis software and Genotyper® software allows allele determination by comparing amplified sample fragments with allelic ladders and internal lane standards. When using an internal lane standard, the calculated lengths of allelic ladder components may differ from those listed in the table. This is due to differences in migration resulting from sequence differences between allelic ladder fragments and internal size standard fragments and is not a matter of concern.

6. Double-click on the Allelic Ladders macro. A plots window will open to display the blue (fluorescein) dye allelic ladders (i.e., Amelogenin, D18S51 and D8S1179) and the green (JOE) dye allelic ladders (i.e., TH01). Confirm that the correct allele designations were assigned to the allelic ladders (Figure 8 in Section VI.G).

   Note: The software uses one ladder sample to determine allele sizes. The macro uses the first ladder sample imported for allele designations. If the POWER macro is run a second time, the software will use the second ladder; if the POWER macro is run a third time, the software will use the third ladder, etc., until all ladders in the project are used. If an allelic ladder fails to be analyzed or if many off-ladder alleles are found in the samples, the samples should be re-analyzed using another ladder from the project.

7. Double-click on the Display Fluorescein Data macro to display the blue dye for all sample injections/lanes. Scroll down to observe and edit as needed.

8. Double-click on the Display JOE Data macro to display the green dye for all sample injections/lanes. Scroll down to observe and edit as needed.

VI.E. Sample Analysis Using the Genotyper® Software and PowerTyper™ S5 Macro (continued)

9. Create the appropriate table by selecting the PowerTable, Make Allele Table or Make Vertical Table macro. The three available table formats are shown below. The PowerTable option allows up to four alleles per sample file. Additional information such as low peak signal or high peak signal is also included. The Allele Table and Vertical Table options include only two alleles per locus. If more than two alleles are present at a locus, the smallest alleles identified are included. The Allele Table format displays the categories (loci) in columns, while the Vertical table format displays the categories in rows. These tables can be customized to fit needs. To save data in tables, go to the Table drop-down menu, highlight "Export to File..." and save the file with the desired name and location. The saved file can be viewed and analyzed using Microsoft® Excel.

PowerTable Format

Sample Info	Sample Comment	Category	Peak 1	Peak 2	Peak 3	Peak 4	Over-flow	Low Signal	Satura-tion	Edited Label	Edited Row

Allele Table Format

Sample Info	Category Allele 1	Category Allele 2	Category Allele 1	Category Allele 2	Category Allele 1	Category Allele 2	Category Allele 1	Category Allele 2

Vertical Table Format

Sample Info	Category	Peak 1	Peak 2

10. Save the analyzed data, go to the File menu and select "Save as".

The PowerTyper™ Macro is a Genotyper® file and can be overwritten if "Save" is used instead of "Save as".

VI.F. Controls

1. Observe the results for the negative control. The negative control should be devoid of amplification products.

2. Observe the results for the 9947A DNA positive control reaction. Compare the control DNA allelic repeat sizes with the locus-specific allelic ladder. The expected 9947A DNA allele designations for each locus are listed in Table 4 (Section IX.A). The 9947A DNA, which is cell line-derived, will show allelic imbalance and imbalance between STR loci.

VI.G. Results

Representative results of the PowerPlex® S5 System are shown in Figure 7. The PowerPlex® S5 Allelic Ladder Mix is shown in Figure 8.

Artifacts and Stutter

Stutter bands are a common amplification artifact associated with STR analysis. Stutter products are often observed one repeat unit below the true allele peak and, occasionally, two repeat units smaller or one repeat unit larger than the true allele peak. Frequently, alleles with a greater number of repeat units will exhibit a higher percent stutter. The pattern and intensity of stutter may differ slightly between primer sets for the same loci.

In addition to stutter peaks, other artifact peaks can be observed at some of the PowerPlex® S5 System loci. Low-level products can be seen in the n–2 and n+2 positions (two bases below and above the true allele peak, respectively) with some loci, such as D18S51.

One or more extra peaks that are not directly related to amplification may be observed at positions 11 bases smaller than TH01 alleles, 1 base smaller than FGA alleles and 1 or 8 bases smaller than Amelogenin alleles. These extra peaks occur when the amplified peaks are particularly intense (high signal level or template amount); the formamide, polymer or capillary was of poor quality; or denaturation was ineffective. One or more extra peaks that are not directly related to amplification may be observed at 73 bp in the fluorescein channel and at 72–76 bp in the JOE channel. See Section VII for more information about how to minimize these artifacts.

Stutter filters can be modified in the PowerPlex® panel and bin sets for the GeneMapper® *ID* software, version 3.2, or the PowerTyper™ Macro if desired. Contact Promega Technical Services (**genetic@promega.com**) for assistance with modifications.

Notes:

1. Peak heights outside the linear range of the instrument may generate artifact peaks due to instrument saturation (i.e., overloading the sample). Bleedthrough (pull-ups) from one color to another may be observed. Saturated signal may also appear as two peaks (split peak).
2. If peak heights are not within the linear range of detection of the instrument, the ratio of stutter peaks to real allele peaks increases, and allele designations become difficult to interpret. The balance of peak heights may also appear less uniform.

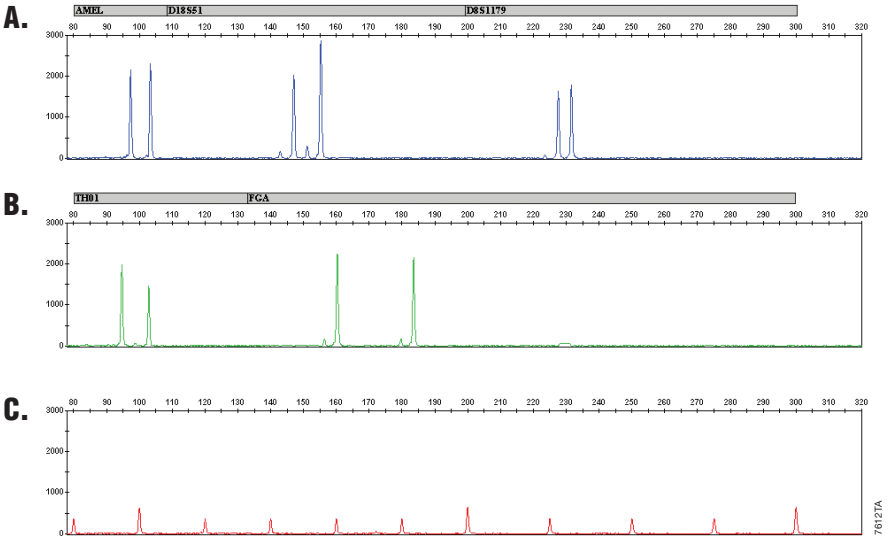

Figure 7. The PowerPlex® S5 System. A single DNA template (250pg) was amplified using the PowerPlex® S5 System. The amplification products were detected using an Applied Biosystems 3130*xl* Genetic Analyzer and a 3kv, 5-second injection. The results were analyzed using GeneMapper® *ID* software, version 3.2. **Panel A.** An electropherogram showing the peaks of the fluorescein-labeled loci: Amelogenin, D18S51 and D8S1179. **Panel B.** An electropherogram showing the peaks of the JOE-labeled loci: TH01 and FGA. **Panel C.** An electropherogram showing the 80bp to 300bp fragments of the Internal Lane Standard 600.

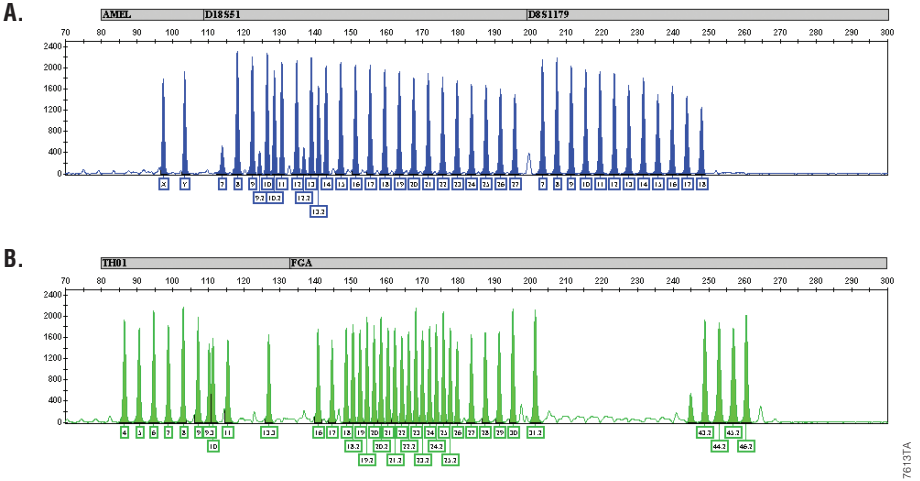

Figure 8. The PowerPlex® S5 Allelic Ladder Mix. The PowerPlex® S5 Allelic Ladder Mix was analyzed using an Applied Biosystems 3130*xl* Genetic Analyzer and a 3kv, 5-second injection. The results were analyzed with the GeneMapper® *ID* software, version 3.2. **Panel A.** The fluorescein-labeled allelic ladder components. **Panel B.** The JOE-labeled allelic ladder components.

VII. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: www.promega.com. E-mail: **genetic@promega.com**

VII.A. Amplification and Fragment Detection

Symptoms	Causes and Comments
Faint or absent allele peaks	Impure template DNA. Because of the small amount of template used, this is rarely a problem. Depending on the DNA extraction procedure used and sample source, inhibitors may be present in the DNA sample.
	Insufficient template. Use the recommended amount of template DNA, increase injection time or voltage, increase cycle number or increase the volume of amplified sample during sample preparation.
	Insufficient enzyme activity. Use the recommended amount of PowerPlex® S5 5X Master Mix, and vortex the 5X Master Mix prior to use.
	Incorrect amplification program. Confirm the amplification program.
	PCR amplification mix was not mixed thoroughly. Vortex mix for 5–10 seconds before dispensing into reaction tubes or plate.
	An air pocket has formed at the bottom of the well. Use a pipette to remove the air pocket, or centrifuge briefly prior to thermal cycling. Centrifuge samples prior to injection on the CE instrument.
	High salt concentration or altered pH. If the DNA template is stored in TE buffer that is not pH 8.0 or contains a higher EDTA concentration, the DNA volume should not exceed 20% of the total reaction volume. Carryover of K^+, Na^+, Mg^{2+} or EDTA from the DNA sample can negatively affect PCR. A change in pH may also affect PCR. Store DNA in TE^{-4} buffer (10mM Tris-HCl [pH 8.0], 0.1mM EDTA) or nuclease-free water.
	Thermal cycler, plate or tube problems. Review the thermal cycling protocols in Section IV.B. We have not tested other reaction tubes, plates or thermal cyclers. Calibrate the thermal cycler heating block, if necessary.
	Primer concentration was too low. Use the recommended primer concentration. Mix the PowerPlex® S5 10X Primer Pair for 15 seconds using a vortex mixer before use.
	Samples were not completely denatured. Heat-denature samples for the recommended time, and cool on crushed ice or in an ice-water bath immediately prior to CE.
	Poor CE injection (ILS 600 peaks also affected). Re-inject the sample. Check the syringe for leakage.Check the laser power.
	Poor-quality formamide was used. Use only Hi-Di™ formamide when analyzing samples.
	The 9947A DNA, which is cell line-derived, will show allelic imbalance and imbalance between STR loci

VII.A. Amplification and Fragment Detection (continued)

Symptoms	Causes and Comments
Extra peaks visible in one or all of the color channels	Contamination with another template DNA or previously amplified DNA. Cross-contamination can be a problem. Use aerosol-resistant pipette tips, and change gloves regularly.
	Samples were not completely denatured. Heat-denature samples for the recommended time, and cool on crushed ice or in an ice-water bath immediately prior to CE.
	Artifacts of STR amplification. PCR amplification of STR systems can result in artifacts that appear as peaks one base smaller than the allele due to incomplete addition of the 3´ A residue. Be sure to perform the 45-minute extension step at 60°C after thermal cycling (Section IV.B).
	High background. Decrease the injection time. See Section V.
	CE-related artifacts ("spikes"). Minor voltage changes or urea crystals passing by the laser can cause "spikes" or unexpected peaks. Spikes sometimes appear in one color but often are easily identified by their presence in more than one color. Re-inject samples to confirm.
	CE-related artifacts (contaminants). Contaminants in the water used with the instrument or to dilute the 10X genetic analyzer buffer may generate peaks in the blue and green dye colors. Use autoclaved water, change vials and wash buffer reservoir.
	Excessive amount of DNA. Amplification of >1ng template can result in a higher number of stutter bands and other artifacts.
	Pull-up or bleedthrough. Pull-up can occur when peak heights are too high or if a poor or incorrect matrix has been applied to the samples. • For the ABI PRISM® 310 Genetic Analyzer, generate a new matrix, and apply it to the samples. For the ABI PRISM® 3100 and 3100-*Avant* and Applied Biosystems 3130 and 3130*xl* Genetic Analyzers, perform a new spectral calibration and rerun the samples. • Instrument sensitivities can vary. Optimize the injection conditions. See Section V.
	Long-term storage of amplified sample in formamide can result in degradation. Repeat sample preparation using fresh formamide.
	The CE polymer was beyond its expiration date, or polymer was stored at room temperature for more than one week.
	Maintain instrumentation on a daily or weekly basis, as recommended by the manufacturer.
	PCR amplification mix was not mixed thoroughly. Vortex mix for 5–10 seconds before dispensing into reaction tubes or plate.
	An air pocket has formed at the bottom of the well. Use a pipette to remove the air pocket, or centrifuge briefly prior to thermal cycling.
Precipitate observed in samples after amplification	A precipitate may form as a result of thermodenaturation of the protein associated with hot start. This precipitate does not affect downstream amplification or capillary performance.

Symptoms	Causes and Comments
Allelic ladder not running the the same as the sample	Allelic ladder and primer pair mix were not compatible. Ensure that the allelic ladder is from the same system as the primer pair mix.
	Poor-quality formamide. Use only Hi-Di™ formamide when analyzing samples.
	Migration of samples changed slightly over the course of a CE run with many samples. This may be due to changes in temperature or the CE column over time. Use a different injection of allelic ladder to determine sizes.
Peak height imbalance	Insufficient template DNA. Use the recommended amount of template DNA. Stochastic effects can occur when amplifying low amounts of template.
	Miscellaneous balance problems. Thaw the 10X Primer Pair Mix and 5X Master Mix completely, and vortex for 5–10 seconds before use. Do not centrifuge the 10X Primer Pair Mix after mixing. Calibrate thermal cyclers and pipettes routinely.
	Impure template DNA. Inhibitors that may be present in forensic samples can lead to allele dropout or imbalance.
	PCR amplification mix was not mixed thoroughly. Vortex for 5–10 seconds before dispensing into the reaction tubes or plate.

VII.B. GeneMapper® *ID* Analysis Software

Symptoms	Causes and Comments
Alleles not called	To analyze samples with GeneMapper® *ID* software, the analysis parameters and size standard must both have "Basic or Advanced" as the analysis type. If they are different, an error is obtained (Figure 9).
	An insufficient number of ILS 600 fragments was defined. Be sure to define at least one ILS 600 fragment smaller than the smallest sample or allelic ladder peak and at least one ILS 600 fragment larger than the largest sample or allelic ladder peak.

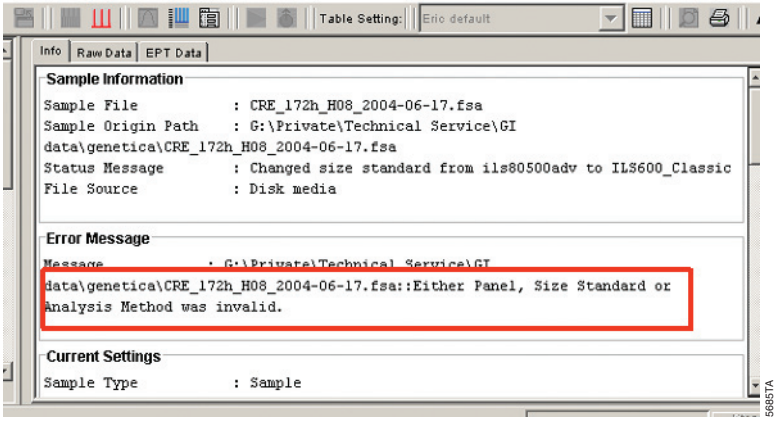

Figure 9. The error message that appears in the GeneMapper® *ID* software when the analysis parameters and the size standard have different analysis types.

Symptoms	Causes and Comments
Alleles not called (continued)	Run was too short, and larger peaks in ILS were not captured. Not all ILS 600 peaks defined in the size standard were detected during the run. • Create a new size standard using the internal lane standard fragments present in the sample. • Rerun samples using a longer run time.
Off-ladder alleles	An allelic ladder from a different run than the samples was used. Re-analyze samples with an allelic ladder from the same run.
	The GeneMapper® *ID* software requires that the allelic ladder be imported from the same folder as the sample. Be sure that the allelic ladder is in the same folder as the sample. Create a new project and re-analyze, as described in Section VI.B or VI.C.
	Panel file selected for analysis was incorrect for the STR system used. Assign correct panel file that corresponds to the system used for amplification.
	The allelic ladder was not identified as an allelic ladder in the sample type column.
	The wrong analysis type was chosen for the analysis method. Be sure to use the HID analysis type.
	The internal lane standard was not properly identified in the sample. Manually redefine sizes of the size standard fragments in the sample.
Size standard not called correctly (Figure 10)	Starting data point was incorrect for the partial range chosen in Section VI.B. Adjust the starting data point in the analysis method. Alternatively, use a full range for the analysis.

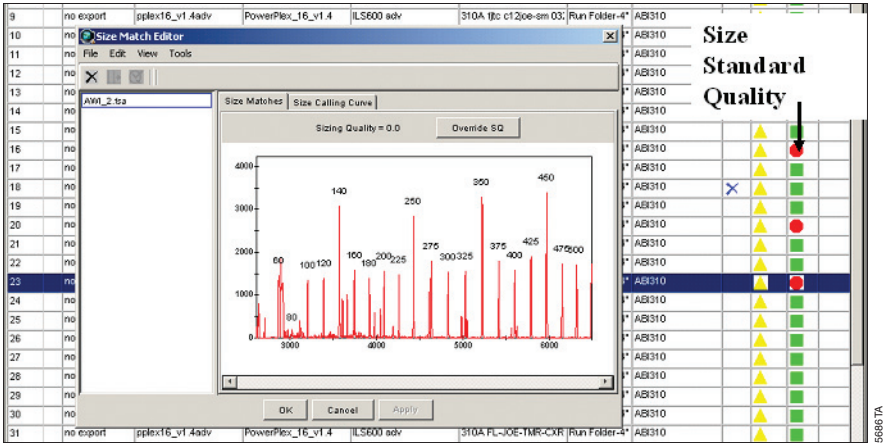

Figure 10. An example showing improper assignment of size standard fragments in the GeneMapper® *ID* software.

Symptoms	Causes and Comments
Size standard not called correctly (Figure 10) (continued)	Extra peaks in advanced mode size standard. Open the size match editor. Highlight the extra peak, select "Edit" and select "delete size label". Select "auto adjust sizes".
	Run was too short, and larger peaks in ILS were not captured. Not all ILS 600 peaks defined in the size standard were detected during the run. • Create a new size standard using the internal lane standard fragments present in the sample. • Rerun samples using a longer run time.
Peaks in size standard missing	If peaks are below threshold, decrease the peak threshold in the analysis method for the red channel to include peaks.
	If peaks are low-quality, redefine the size standard to skip these peaks.
Error message: "Either panel, size standard, or analysis method is invalid"	The size standard and analysis method were not in the same mode ("Classic" vs. "Basic or Advanced"). Be sure both files are set to the same mode, either Classic or Basic or Advanced mode.
No alleles called, but no error message appears	Panel was not selected for sample. In the Panel column, select the appropriate panel set for the STR system that was used.
	No size standard was selected. In the size standards column, be sure to select the appropriate size standard.
	Size standard was not correctly defined, or size peaks were missing. Redefine size standard to include only peaks present in your sample. Terminating analysis early or using short run times will cause larger ladder peaks to be missing. This will cause your sizing quality to be flagged as "red", and no allele sizes will be called.
Error message: "Both the Bin Set used in the Analysis Method and the Panel must belong to the same Chemistry Kit".	The bin set assigned to the analysis method may have been deleted. In the GeneMapper® Manager, select the Analysis Methods tab, and open the analysis method of interest. Select the Alleles tab, and select an appropriate bin set.
Significantly raised baseline	• Poor spectral calibration for the ABI PRISM® 3100 and 3100-*Avant* Genetic Analyzers and Applied Biosystems 3130 and 3130*xl* Genetic Analyzers. Perform a new spectral calibration, and rerun the samples. • Poor matrix for the ABI PRISM® 310 Genetic Analyzer. Rerun and optimize the matrix.
	Use of Classic mode analysis method. Use of Classic mode analysis for samples can result in baselines with more noise than those analyzed using the Basic or Advanced mode analysis method. Advanced mode analysis methods and size standards are recommended.

VII.B. GeneMapper® *ID* Analysis Software (continued)

Symptoms	Causes and Comments
Red bar appears during analysis of samples, and the following error message appears when data are displayed: "Some selected sample(s) do not contain analysis data. Those sample(s) will not be shown".	If none of the samples had matrices applied when run on the ABI PRISM® 310 Genetic Analyzer, no data will be displayed. Apply a matrix file during analysis in the GeneMapper® *ID* software and re-analyze.
Error message after attempting to import panel and bin files: "Unable to save panel data: java.SQLEException: ORA-00001: unique constraint (IFA.CKP_NNN) violated".	There was a conflict between different sets of panel and bin files. Delete all panels and bins, and re-import in a different order.
Allelic ladder peaks are labeled off-ladder	GeneMapper® *ID* software was not used, or microsatellite analysis settings were used instead of HID analysis settings. GeneMapper® software does not use the same algorithms as GeneMapper® *ID* software and cannot correct for sizing differences using the allelic ladder. Promega recommends GeneMapper® *ID* software for analysis of PowerPlex® reactions. If using GeneMapper® *ID* software, version 3.2, be sure that the analysis method selected is an HID method. This can be verified by opening the analysis method using the GeneMapper® Manager, then selecting the General tab. The analysis type cannot be changed. If the method is not HID, it should be deleted and a new analysis method created.

VII.C. PowerTyper™ S5 Macro

Symptoms	Causes and Comments
File does not open on your computer	Genotyper® software was not installed. Be certain that the Genotyper® software, version 2.5 (Macintosh®) or version 3.6 or higher (Windows NT®), is installed.
	Incorrect version of Genotyper® software. The PowerTyper™ S5 Macro will not work with Genotyper® software versions prior to version 2.5.
	The file was corrupted during download. Download the file again.
Error message: "Could not complete the "Run Macro" command because no dye/lanes are selected"	Allelic ladder sample files were not identified. Be certain the sample info or color info column for each lane containing PowerPlex® S5 Allelic Ladder Mix contains the word "ladder". The macro uses the word "ladder" to identify sample files containing allelic ladder.
	All dye colors were not imported. For Genotyper® software, versions 2.5 and 3.5 or higher, set preferences (in the Edit menu) to import the blue, green and red colors.

Symptoms	Causes and Comments
Error message: "Could not complete the "Run Macro" command because the labeled peak could not be found"	Peak heights for one or more of alleles in the allelic ladder sample file were below 150RFU. The allelic ladder categories are defined as having a minimum peak height of 150RFU. If peak heights of ladder alleles are below 150RFU, the software will not be able to locate the allele peak. Rerun the allelic ladder using more sample or longer injection time to assure peak heights above 150RFU.
	CE spikes in the allelic ladder were identified as alleles by the macro. Use a different injection of allelic ladder.
	TH01 9.3 and 10 alleles were not separated when using heavy smoothing in the GeneScan® analysis parameters. Use light smoothing in the GeneScan® analysis parameters.
	The base-pair size of alleles in the allelic ladder are outside of the defined category range. Be sure the internal lane standard fragments are correctly sized. Redefine the internal lane standard fragments, and re-analyze the sample using GeneScan® software. Compare the size of the smallest allele in the allelic ladder with the base-pair size and range listed in the categories for the same alleles. If necessary, increase the category start range (in the category window) to greater than ±6bp, and save the macro under a new name.
	Allelic ladder peaks were too high, causing stutter peaks to be called as allele peaks. Use a shorter injection time, decrease the amount of allelic ladder used or re-analyze the allelic ladder sample using increased peak amplitude thresholds in the GeneScan® analysis parameters.
	Allelic ladder data were not compatible with the PowerTyper™ file used. Confirm that the PowerTyper™ Macro file matches the allelic ladder being used.
The plots window or allele table does not display all data	The macros were not run in the proper order. Use the POWER or POWER 20% Filter macro option.
	All three dye colors were not imported. For Genotyper® software, versions 2.5 and 3.5 or higher, set preferences (in the Edit menu) to import the blue, green and red colors.
The Check ILS macro displays an empty plot window	All three dye colors were not imported. For Genotyper® software, versions 2.5 and 3.5 or higher, set preferences (in the Edit menu) to import the blue, green and red colors.
Off-ladder peaks	Migration of samples changed slightly over the course of a CE run with many samples. This may be due to changes in temperature or the CE column over time. Use a different injection of allelic ladder to determine sizes in the PowerTyper™ S5 Macro. Do not use the first injection on a new column for the ladder sample.
	The base-pair size of alleles was incorrect because incorrect fragment sizes were assigned to the internal lane standard. Confirm that internal lane standard fragments are assigned correctly. Re-analyze sample using GeneScan® software, and redefine internal lane standard fragments.

VIII. References

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Additional STR references can be found at: **www.promega.com/geneticidentity/**

IX. Appendix

IX.A. Advantages of Using the Loci in the PowerPlex® S5 System

The loci included in the PowerPlex® S5 System (Tables 2 and 3) have been selected to include four of the current seven ENFSI loci (12–24) and four of the current CODIS loci. Additionally, the Amelogenin locus is included in the PowerPlex® S5 System to allow gender identification of each sample. Table 4 lists the PowerPlex® S5 System alleles revealed in commonly available standard DNA templates.

Table 2. The PowerPlex® S5 System Locus-Specific Information.

STR Locus	Label	Chromosomal Location	GenBank® Locus and Locus Definition	Repeat Sequence[1] 5´→ 3´
D8S1179	FL	8q	NA	TCTA Complex (25)
D18S51	FL	18q21.3	HUMUT574	AGAA (25)
Amelogenin[2]	FL	Xp22.1–22.3 and Y	HUMAMEL, human Y chromosomal gene for Amelogenin-like protein	NA
FGA	JOE	4q28	HUMFIBRA, human fibrinogen alpha chain gene	TTTC Complex (25)
TH01	JOE	11p15.5	HUMTH01, human tyrosine hydroxylase gene	AATG (25)

[1]The August 1997 report (26,27) of the DNA Commission of the International Society for Forensic Haemogenetics (ISFH) states, "1) for STR loci within coding genes, the coding strand shall be used and the repeat sequence motif defined using the first possible 5´ nucleotide of a repeat motif; and 2) for STR loci not associated with a coding gene, the first database entry or original literature description shall be used".

[2]Amelogenin is not an STR but displays a 103-base, X-specific band and a 109-base, Y-specific band.

FL = fluorescein

JOE = 6-carboxy-4´,5´-dichloro-2´,7´-dimethoxyfluorescein

Table 3. The PowerPlex® S5 System Allelic Ladder Information.

STR Locus	Label	Size Range of Allelic Ladder Components[1] (bases)	Repeat Numbers of Allelic Ladder Components
D8S1179	FL	208–252	7–18
D18S51	FL	123–199	8–10, 10.2, 11–13, 13.2, 14–27
Amelogenin[2]	FL	103, 109	X, Y
FGA	JOE	148–270	16–18, 18.2, 19, 19.2, 20, 20.2, 21, 21.2, 22, 22.2, 23, 23.2, 24, 24.2, 25, 25.2, 26–30, 31.2, 43.2, 44.2, 45.2, 46.2
TH01	JOE	93–132	4–9, 9.3, 10–11, 13.3

[1]When using an internal lane standard, such as the Internal Lane Standard 600, the calculated sizes of allelic ladder components may differ from those listed. This occurs because different sequences in allelic ladder and ILS components may cause differences in migration. The dye label also affects migration of alleles.

[2]Amelogenin is not an STR but displays a 103-base, X-specific band and a 109-base, Y-specific band.

Table 4. The PowerPlex® S5 System Allele Determinations in Commonly Available Standard DNA Templates.

	Standard DNA Templates[1]		
STR Locus	**K562**	**9947A**	**9948**
D8S1179	12, 12	13, 13	12, 13
D18S51	15, 16	15, 19	15, 18
Amelogenin	X, X	X, X	X, Y
FGA	21, 24	23, 24	24, 26
TH01	9.3, 9.3	8, 9.3	6, 9.3

[1]Information on strains 9947A, 9948 and K562 is available online at: locus.umdnj.edu/nigms Strain K562 is available from the American Type Culture Collection: www.atcc.org (Manassas, VA). Information about the use of 9947A and 9948 DNA as standard DNA templates can be found in reference 28.

We have carefully selected primers to avoid or minimize artifacts, including those associated with *Taq* DNA polymerase, such as repeat slippage and terminal nucleotide addition. Repeat slippage (29,30), sometimes called "n–4 bands", "stutter" or "shadow bands", is due to the loss of a repeat unit during DNA amplification, somatic variation within the DNA, or both. The amount of this artifact observed depends primarily on the locus and DNA sequence being amplified.

Terminal nucleotide addition (31,32) occurs when *Taq* DNA polymerase adds a nucleotide, generally adenine, to the 3´ ends of amplified DNA fragments in a template-independent manner. The efficiency with which this occurs varies with different primer sequences. Thus, an artifact band one base shorter than expected (i.e., missing the terminal addition) is sometimes seen. We have modified primer sequences and added a final extension step of 60°C for 45 minutes (33) to the amplification protocol to provide conditions for essentially full terminal nucleotide addition when recommended amounts of DNA template are used.

The presence of microvariant alleles (alleles differing from one another by lengths other than the repeat length) complicates interpretation and assignment of alleles. There appears to be a correlation between a high degree of polymorphism, a tendency for microvariants and increased mutation rate (34,35). FGA and D18S51 display numerous, relatively common microvariants.

IX.B. DNA Extraction and Quantitation Methods

The DNA IQ™ System (Cat.# DC6700) is a DNA isolation and quantitation system designed specifically for forensic and paternity samples (36). This novel system uses paramagnetic particles to prepare clean samples for STR analysis easily and efficiently and can be used to extract DNA from stains or liquid samples, such as blood or solutions. The DNA IQ™ Resin eliminates PCR inhibitors and contaminants frequently encountered in casework samples. With larger samples, the DNA IQ™ System delivers a consistent amount of total DNA. The system has been used to isolate and quantify DNA from routine sample types including buccal swabs, stains on FTA® paper and liquid blood. Additionally, DNA has been isolated from casework samples such as tissue, differentially separated sexual assault samples and stains on support materials. The DNA IQ™ System has been tested with the PowerPlex® Systems to ensure a streamlined process. See Section IX.F for ordering information.

For applications requiring human-specific DNA quantification, the Plexor® HY System (Cat.# DC1001, DC1000) has been developed (37). See Section IX.F for ordering information.

The DNA IQ™ System has been fully automated on the Beckman Coulter Biomek® 2000 Laboratory Automation Workstation (38), Biomek® 3000 Laboratory Automation Workstation (39) and Tecan Freedom EVO® Liquid Handler (40). In addition, the DNA IQ™ Reference Sample Kit for Maxwell® 16 (Cat.# AS1040) and DNA IQ™ Casework Sample Kit for Maxwell® 16 are available (41,42). For information on automation of laboratory processes on automated workstations, contact your local Promega Branch Office or Distributor (contact information available at: **www.promega.com/worldwide/**) or e-mail: **genetic@promega.com**

IX.C. The Internal Lane Standard 600

The Internal Lane Standard (ILS) 600 contains 22 DNA fragments of 60, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 550 and 600 bases in length (Figure 11). For PowerPlex® S5 analyses only ILS 600 fragments smaller than 350bp need to be detected. Each fragment is labeled with carboxy-X-rhodamine (CXR) and may be detected separately in the presence of PowerPlex® S5-amplified material. The ILS 600 is designed for use in each CE injection to increase precision in analyses when using the PowerPlex® S5 System.

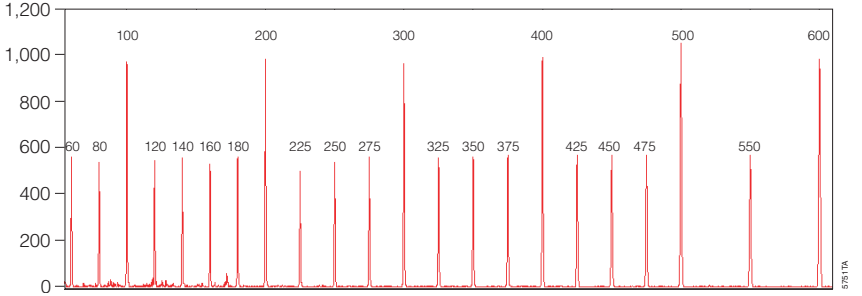

Figure 11. Internal Lane Standard 600. An electropherogram showing the fragments of the Internal Lane Standard 600.

IX.D. Preparing the PowerPlex® S5 System PCR Amplification Mix

A worksheet to calculate the required amount of each PCR amplification mix component is provided in Table 5. Determine the number of reactions to be set up. This should include positive and negative control reactions. Add 1 or 2 reactions to this number to compensate for pipetting error. Multiply the volume (µl) per reaction by the total number of reactions to obtain the final PCR amplification mix volume (µl).

Table 5. PCR Amplification Mix for PowerPlex® S5 System Reactions.

PCR Amplification Mix Component	Volume Per Reaction	×	Number of Reactions	=	Final Volume (µl)
Water, Amplification Grade[1]	µl	×		=	
PowerPlex® S5 5X Master Mix	5.0µl	×		=	
PowerPlex® S5 10X Primer Pair Mix	2.5µl	×		=	
Per tube					
template DNA volume (0.25–0.50ng)	up to 17.5µl	×		=	
total reaction volume	25µl	×		=	

[1]The total volume of PCR amplification mix volume and template DNA should be 25µl.

IX.E. Composition of Buffers and Solutions

TE^{-4} buffer (10mM Tris-HCl, 0.1mM EDTA [pH 8.0])

2.21g	Tris base
0.037g	EDTA ($Na_2EDTA \cdot 2H_2O$)

Dissolve Tris base and EDTA in 900ml of deionized water. Adjust to pH 8.0 with HCl. Bring the final volume to 1 liter with deionized water.

IX.F. Related Products

Fluorescent STR Multiplex Systems

Product	Size	Cat.#
PowerPlex® 16 System	100 reactions	DC6531
	400 reactions	DC6530
PowerPlex® 16 BIO System	100 reactions	DC6541
	400 reactions	DC6540
PowerPlex® Y System	50 reactions	DC6761
	200 reactions	DC6760
PowerPlex® ES System	100 reactions	DC6731
	400 reactions	DC6730

Not for Medical Diagnostic Use.

Accessory Components

Product	Size	Cat.#
PowerPlex® Matrix Standards, 310*	50µl (each dye)	DG4640
PowerPlex® Matrix Standards, 3100/3130*	25µl (each dye)	DG4650
9947A DNA*	250ng	DD1001
Internal Lane Standard 600**	150µl	DG1071
Water, Amplification Grade**	6,250µl (5 × 1,250µl)	DW0991

*Not for Medical Diagnostic Use.
**For Laboratory Use.

Sample Preparation Systems

Product	Size	Cat.#
DNA IQ™ System**	100 reactions	DC6701
	400 reactions	DC6700
Differex™ System*	50 samples	DC6801
	200 samples	DC6800
Maxwell® 16 Instrument**	each	AS2000
DNA IQ™ Reference Sample Kit for Maxwell® 16***	48 preps	AS1040
DNA IQ™ Casework Sample Kit for Maxwell® 16***	48 preps	AS1210
Plexor® HY System*	200 determinations	DC1001
	800 determinations	DC1000
Slicprep™ 96 Device**	10 pack	V1391

*Not for Medical Diagnostic Use.
**For Laboratory Use.
***For Research Use Only. Not for use in diagnostic procedures.

ART® Aerosol-Resistant Tips

Product	Volume	Size (tips/pack)	Cat.#
ART® 10 Ultramicro Pipet Tip	0.5–10µl	960	DY1051
ART® 20E Ultramicro Pipet Tip	0.5–10µl	960	DY1061
ART® 20P Pipet Tip	20µl	960	DY1071
ART® GEL Gel Loading Pipet Tip	100µl	960	DY1081
ART® 100 Pipet Tip	100µl	960	DY1101
ART® 100E Pipet Tip	100µl	960	DY1111
ART® 200 Pipet Tip	200µl	960	DY1121
ART® 1000E Pipet Tip	1,000µl	800	DY1131

[a]STR loci are the subject of U.S. Pat. No. RE 37,984, German Pat. No. DE 38 34 636 C2 and other patents issued to the Max-Planck-Gesellschaft zur Förderung der Wissenschaften, e.V., Germany. The development and use of STR loci are covered by U.S. Pat. No. 5,364,759, Australian Pat. No. 670231 and other pending patents assigned to Baylor College of Medicine, Houston, Texas.

Patents for the foundational PCR process, European Pat. Nos. 201,184 and 200,362, expired on March 28, 2006. In the U.S., the patents covering the foundational PCR process expired on March 29, 2005.

[b]This product is sold under licensing arrangements with the USB Corporation. The purchase price of this product includes limited, nontransferable rights under U.S. Patent Application Serial Number 11/171,008 owned by the USB Corporation to use only this amount of the product to practice the claims in said patent solely for activities of end users within the fields of life science research and forensic analysis of genetic material relating to, or obtained as the result of, criminal investigations or disaster sites conducted either by or for a governmental entity, or for use in or preparation for legal proceedings, as well as the compilation and indexing of the results of such analysis, and also analysis for parentage determination (the "Forensic and Genetic Identity Applications Field"). The Forensic and Genetic Identity Applications Field specifically excludes tissue typing related to transplantation or other medical procedures. Further licensing information may be obtained by contacting the USB Corporation, 26111 Miles Road, Cleveland, OH 44128.

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